HORMONAL REGULATION OF THE DISTAL RETINAL PIGMENT OF PALAEMONETES

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There have been several investigations (Parker, 1897; Welsh, 1930; Bennitt, 1932; Kleinholz, 1936) of the control of the migration of the distal retinal pigment of the prawn, *Palaemonetes.* Bennitt demonstrated that when one eye of *Palaemonetes* was covered and the other eye illuminated, both eyes assumed to a greater or lesser extent the light-adapted state. This suggested a hormonal control of the retinal pigments. Later, Kleinholz (1936) induced the distal retinal pigment of *Palaemonetes* to assume the light-adapted position by injecting eye-stalk extract into dark-adapted prawns, thus suggesting the existence of a light-adapting hormone whose source was the eye-stalk. That this was the normal controlling mechanism was indicated by the fact that eye-stalks of light-adapted animals were more bountifully supplied with this factor than those of dark-adapted ones.

The results of the experiments of Brown, Fingerman and Hines (1952) and Brown, Webb and Sandeen (1952) in which dark-adapted prawns are exposed to 250 ft.-c. light flashes both with and without earlier conditioning stimuli have not only given strong support to the hypothesis that there is a light-adapting hormone normally active in regulating the position of the distal retinal pigment of *Palaemonetes,* but have also indicated the action of a dark-adapting hormone. Prawns kept in darkness overnight exhibit quite a different rate of re-dark-adaptation when returned to darkness following a one-minute, 250 ft.-c. light exposure early in the morning than do prawns which earlier the same day had received the normal stimulus of dawn illumination before being placed in darkness and later submitted to the one-minute light exposure. Such a difference was postulated to be due to different degrees of availability of a dark-adapting factor.

The present experiments were undertaken: (1) to establish more definitely the presence of a dark-adapting hormone, (2) to determine the sources of the light- and dark-adapting hormones within *Palaemonetes,* and (3) to elucidate further the normal roles of these hormones in light- and dark-adaptation.

Materials and Methods

For the following experiments specimens of the common prawn, *Palaemonetes vulgaris,* were usually collected daily from the Eel Pond in Woods Hole, Massachusetts. In the laboratory the stock supply of animals was kept in aquaria in running sea-water from which the animals for each experiment were randomly taken without regard to size or sex. The experiments were conducted during the months of June and July.

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The influence of the various experimental procedures on the distal retinal pigment in the eyes of these prawns was observed through the use of a method of direct measurement of the position of this pigment in the intact, living animal (Sandeen and Brown, 1952). The method consisted essentially of holding a prawn on the stage of a dissecting microscope and viewing the eye under a relatively high magnification using transmitted light. With an ocular micrometer the width of the transparent area, which is a direct function of the degree of light-adaptation, can be measured from the cornea to the distal margin of the pigment. Since the animals varied considerably in size, the degree of light-adaptation was always expressed as a ratio of this width to the total distance from corneal surface to the proximal edge of the retina using arbitrarily as a marker the black spot, or ocellus, which is apparent on the dorsal aspect of the eye-stalk. This ratio will be referred to as the distal pigment index.

In all experiments in which *Palaemonetes* were placed in the dark room or exposed to various light intensities, white enamelled pans with a bottom diameter of approximately 7 inches were used. Sea water was placed in the containers to a depth of about 1½ inches. To prevent overcrowding no more than 12 to 14 animals were ever placed in a single pan. The various illuminations which were used were obtained with incandescent lamps placed at adjusted distances directly above the animals. The resultant illuminations were measured with a Weston photometer.

In the experiments in which prawns were injected with extracts of various organs the extracts were prepared in the following manner. The organs were removed with the aid of a dissecting microscope, from normal animals taken from the stock supply, and transferred to a small container of sea water. When a sufficient number of the organs had been obtained they were placed with a minimum of water in a glass mortar with a finely ground surface and while still moist were triturated as completely as possible with a glass pestle. Sea water was then added to make up the desired concentration, mixed thoroughly, and the extract drawn into a one ml. hypodermic syringe graduated in hundredths. The whole procedure was carried out very rapidly and the extracts used immediately. In no case did more than 30 minutes elapse between the beginning of the dissections and the injections.

Comparison of the Responses of One-Eyed and Normal Animals

In the first series of experiments the influence of the removal of one eye on the distal retinal pigment of the remaining eye was determined. In each of six experiments five *Palaemonetes* were taken at random from the stock supply and placed in a small amount of sea water in a white enamelled pan at an illumination of 25 to 50 foot-candles. One eye-stalk was carefully removed with a pointed scalpel under a dissecting microscope and the eye-stub cauterized immediately. The position of the distal pigment of each eye to be observed in the experiment was determined prior to the operation and then at 5, 10, 15, 30, 60, 90, 120 and 240 minutes following the operation. The first three of these experiments were conducted in the morning and the last three in the early afternoon. No significant difference in responses was found for these two times of day.

The average distal pigment index of the five animals in each experiment was calculated for each time that a determination was made. These averages were used to calculate a final distal retinal pigment index for each time that a reading was

made for all six experiments. Therefore, each final index at any given time represents an average of the condition for 30 animals. The final average indices of the six experiments were used to prepare Figure 1(A). It can be seen that the distal pigment index drops from 0.18 to 0.16 in 30 minutes and remains at this lower level for the duration of the experiment, a period of four hours. It had previously been determined (Sandeen and Brown, 1952) that at this illumination the distal pigment index of normal animals has a value ranging between 0.18 and 0.20. This is represented in Figure 1(A) by the broken line above the curve just described. Thus, under conditions of equal illumination the distal retinal pigment of *Palaemonetes* with only one eye-stalk is maintained at a lesser degree of light-adaptation than that in normal animals with their two eye-stalks.

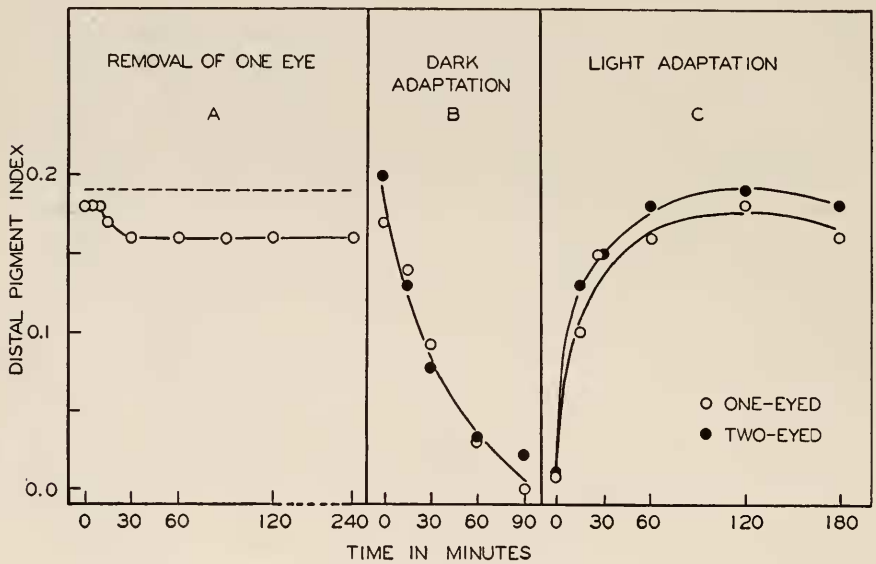

FIGURE 1. A. Change in the degree of light adaptation of the distal retinal pigment following removal of contralateral eyestalk. B. Comparison of rates of dark-adaptation in one-stalked and normal prawns. C. Comparison of rates and degrees of light-adaptation in one-stalked and normal prawns.

In a second series of experiments the rate of dark-adaptation of one-eyed prawns was compared with that of normal prawns. Approximately twenty-four hours before each experiment specimens of *Palaemonetes* were taken from the stock supply of animals and one eye of each was removed and the eye-stub cauterized. These animals were then returned to an aquarium with running sea water until the experiment was conducted the following day.

In each of the two experiments which were performed, 10 one-eyed *Palaemonetes* were placed in each of five pans. Similarly, normal animals were taken at random from the stock supply and 10 were placed in each of five pans. The distal pigment indices of 10 one-eyed animals and 10 normal animals in one of the pans of each group were determined. These two pans together with the others in each group

were then placed in the dark room. In the first experiment distal pigment indices of one-eyed and normal *Palaemonetes* were determined at 15, 30, 60 and 90 minutes after being put into the dark. In the second experiment distal pigment indices were determined only at 30, 60, and 90 minutes. At each of these times one container from each group was removed from the dark room and the distal pigment indices of the animals were determined. Following the determination these animals were discarded; a different container of animals was used for each successive determination.

The average distal pigment index for 10 animals of each group at each time that a determination was made was calculated. Average values were then obtained for both experiments and these were used to prepare Figure 1(B). Since a distal pigment index determination was not made at 15 minutes in the second experiment, the average index shown in the graph is that obtained in the first experiment.

It can be seen from Figure 1(B) that there is no significant difference between the rates of dark-adaptation of these two groups of animals. The presence of one eye-stalk is sufficient to permit an animal to dark-adapt at the normal rapid rate.

In a third series of experiments the rate of light adaptation of one-eyed animals was compared with that of normal animals. In each experiment a group of six pans containing 10 normal animals each and a group of six similar pans with 10 one-eyed animals in each were left in darkness overnight and then brought abruptly into an illumination of 250 ft.-c. where the course of light-adaptation of the animals was followed.

The distal pigment indices of 10 normal animals and 10 one-eyed animals were determined at the time the two groups were brought into the light and at 15, 30, 60, 120 and 180 minutes thereafter. The average value at each time of index determination for three such experiments was calculated. Since the determinations in the first of the three experiments were not continued beyond 120 minutes, the average value for 180 minutes was obtained from the last two experiments.

The average distal pigment indices obtained from the three experiments for each time that a determination was made were used to prepare Figure 1(C). It can be seen from this graph that the rate of light-adaptation and the final degree achieved with one-eyed animals are less than that with normal animals. The distal pigment index reached by the one-eyed animals is quite comparable to that seen following the removal of one eye from normal animals in light. It appears, therefore, that both eye-stalks are essential for the normal rate and degree of light-adaptation of each eye under these conditions of illumination.

Influence of a Brief Light Exposure

The extensive light-adaptation in the dark which results from the interruption of a long dark period by a one-minute exposure to light at 250 ft.-c., described elsewhere (Brown, Fingerman and Hines, 1952), suggested the operation of a light-adapting hormone. It appears that an adequate stimulus for secretion of such a substance is a brief, bright, light flash and that the activity of the substance long outlasts the duration of the original brief stimulus. Prawns with a single eyestalk and in darkness responded to a one-minute, 250 ft.-c. flash by substantially less light-adaptation than that seen in normal prawns subjected to a similar stimulus.

It was desired further to determine whether a second substance might be operating in dark-adaptation as postulated by Brown, Fingerman and Hines (1952).

Since a change from darkness to light is evidently an adequate stimulus for the release of the light-adapting hormone it seemed reasonable that a change from light to darkness would be a normal stimulus causing secretion of a dark-adapting hormone. In order to test this possibility an experiment was designed in which responses to a brief light flash were compared following different durations of dark periods.

Palaemonetes were taken at random from the stock supply and distributed among six groups of five white enamelled pans. Approximately 12 animals were put into each pan. The first group of animals constituted the control group. Distal pig-

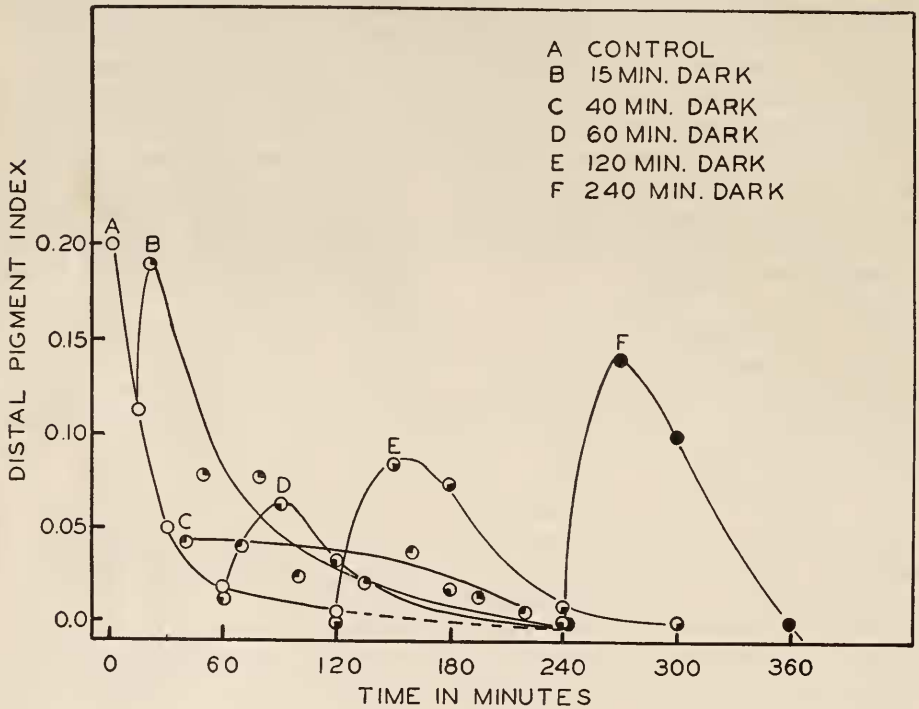

FIGURE 2. Responses of the distal pigment to a one-minute, 250 ft.-c. light flash after different intervals in darkness.

ment indices of 10 animals from one of the pans of this group were determined at 9 A.M. and the remaining containers were placed immediately in darkness. The course of dark-adaptation was followed by removing a pan from darkness and determining distal pigment indices of 10 animals after 15, 30, 60 and 120 minutes.

The remaining five groups of pans were also placed in darkness at approximately 9 A.M. At 15 minutes one group of five pans was subjected to a one-minute, 250 ft.-c., flash of light. Immediately following the flash the distal pigment indices of 10 animals from one pan were determined. The course of dark-adaptation of this group was followed by determining distal pigment indices of 10 animals after 30, 60, 120, and 180 minutes. Similarly, of the other groups, one was dark-

adapted for 40 minutes, one for 60 minutes, one for 120 minutes and the last for 240 minutes. At the end of each of these periods the group of animals was treated in a similar manner to that described for the 15-minute group.

In all the groups for each time that a determination was made an average distal pigment index of the 10 animals was determined. These average indices were used to prepare Figure 2 where the distal pigment index is plotted against time in minutes. In Figure 2 zero time represents that time at which all groups were put into darkness for the first time.

It is apparent from this figure that both the amount of light-adaptation induced by the one-minute flash and the rate of subsequent re-dark-adaptation are influenced by the duration of the dark period. By examination of curves C, D, E, and F, representing 40, 60, 120 and 240 minutes of exposure to darkness prior to the light flash, it can be seen that the longer the period in darkness the greater the extent of light-adaptation resulting from the flash and the more rapid the subsequent rate of dark-adaptation. However, it can also be seen that 15 minutes of exposure to darkness is not sufficiently long to yield a result which is entirely consistent with this generalization. This group light-adapted during the first 15 minutes following the flash to a state almost equivalent to the fully light-adapted state shown by all groups when they were removed from the aquaria at the beginning of the experiment, as illustrated by the initial index of the control group. The rate of dark-adaptation was, however, somewhat less than that shown by the controls.

These results strongly support the hypothesis of the operation of two hormones, one inducing light-, and the other, dark-adaptation. Since greater degrees of light-adaptation result from a one-minute flash of light as the animals are left longer in darkness, it would appear that the ability to secrete the light-adapting principle increases, at least for a few hours, in darkness. On the other hand, the increase in the rate of dark-adaptation with increasing time in darkness can be explained in terms either of an increase in ability to secrete the dark-adapting principle in response to return to darkness or to the presence of a higher titer of this material in the blood for the normal maintenance of the dark-adapted state.

Injection Experiments

In an attempt to obtain some information regarding the sources of the substances which function in the light- and dark-adaptation of *Palaemonetes* distal pigment, experiments were designed in which extracts of eyestalks and of central nervous organs were injected into animals under various conditions.

The first type of experiment consisted of injecting extracts of eyestalks of *Palaemonetes* into animals which had been previously dark-adapted for a minimum of three hours. These animals were taken at random from the stock supply and five were placed in each of four white enamelled pans partially filled with sea water. The extract was prepared by triturating freshly removed eyestalks in a sufficient quantity of sea water to yield such a final concentration that each animal, receiving an injection of 0.02 ml., received the equivalent of one eyestalk. The extracts were centrifuged. The animals were injected in the dark-room under a red photographic light which had previously been tested and shown to produce no light-adaptation. At 30, 60, 120 and 240 minutes one pan of animals was removed from the darkroom and the distal pigment indices of the five animals determined. An

average distal pigment index was calculated for each time that a reading was made. Two experiments of this sort were performed and the results averaged. The averages of the two experiments were used to prepare Figure 3.

It can be seen from Figure 3 that the injection of eyestalk extract produces light-adaptation comparable to that produced by the interruption of a dark period by a one-minute, 250 ft.-c. flash of light. The maximum degree of light-adaptation achieved occurs in 30–60 minutes following injection. The dark-adaptation which followed was not quite complete at the end of 180 minutes.

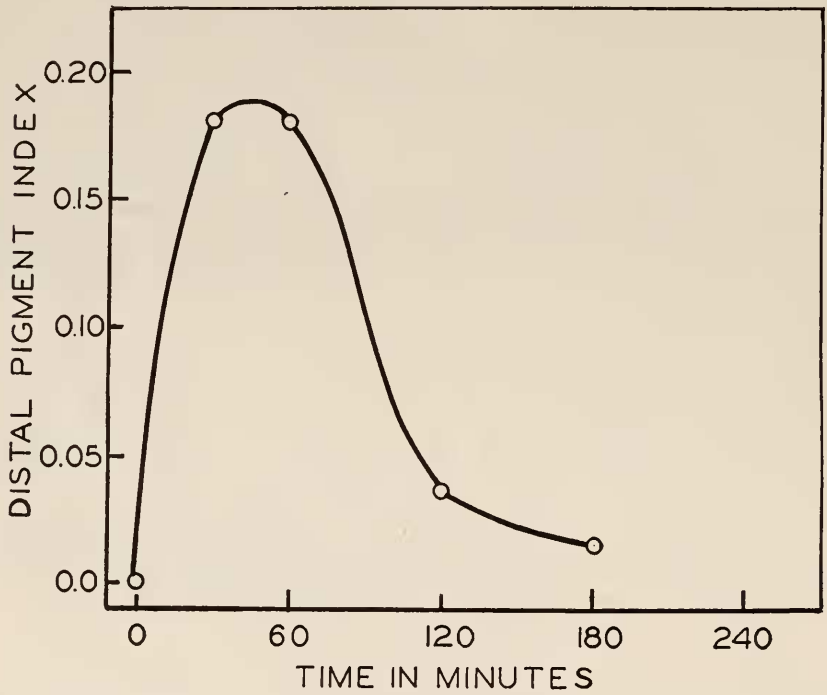

FIGURE 3. Response of dark-adapted prawns in darkness to injection of an extract of eyestalks.

Preliminary experiments in which sea-water extracts of various central nervous organs were injected into similarly dark-adapted *Palaemonetes* yielded inconsistent results. In some cases no light-adaptation was obtained while in others distal pigment indices of the order of 0.03 were obtained, suggesting the presence of light-adapting substance in nervous tissue.

Another method of assay of the extracts was used. A study was made of the influence of injection of extracts of eyestalks and of central nervous organs on the response of dark-adapted prawns to a brief exposure to bright light. For each experiment *Palaemonetes* were taken from the stock supply and distributed among three groups of five pans with five animals in each pan. These animals were all placed in the darkroom overnight and then about 6 the next morning brought abruptly into an illumination of 250 ft.-c. for a period of 20 minutes. During this

period of light exposure one group received injection of an extract of the brain, connectives and ventral cord. The second group received extract of eyestalk, and the third received only sea water. Each animal was given a dose of 0.02 ml.

The extracts of the central nervous system were prepared by dissecting the brain, circumesophageal connectives, and thoracic and abdominal cords from normal light-adapted animals and extracting them in sea water in such an amount that an animal receiving a dose of 0.02 ml. received the equivalent of half a total nervous system. The extract was centrifuged. The extract of the eyestalks was prepared as previously described, such that each animal received one eyestalk or half the equivalent of the complement of a normal animal.

Two experiments of this sort were performed. In the first, distal pigment indices of five animals in one of the pans were determined immediately following the exposure to light while the rest of the containers were placed in the darkroom. Successive determinations of distal pigment indices were made at 30, 60, 120 and 180 minutes, using a different pan of animals for each determination. In the second experiment determinations of the distal pigment indices were made at 30, 60, 120, 180 and 240 minutes from the time the pans were returned to darkness. Averages of the distal pigment indices of five animals for each time that a determination was made were calculated and average values for the two experiments were obtained. Since the initial and 240-minute determinations were made in only one of the two experiments, the average obtained in one experiment was used as the definitive one.

The average distal pigment indices for the two experiments were used to prepare Figure 4. It can be seen from this figure that, compared with the sea-water controls, extracts of the central nervous system as well as those of the eyestalks supplement the amount of light-adaptation which occurs as a result of the exposure to light. Furthermore, there is a significant increase in the subsequent rate of dark-adaptation over that seen for the controls. The extracts, therefore, appeared to contain both light- and dark-adapting principles.

In an effort to determine whether the two hormones are differentially distributed within the central nervous system further experiments were designed in which extracts of various parts of the nervous system were compared with extracts of the eyestalks. In preliminary experiments of this sort it was found that extracts of the abdominal cord, thoracic cord, circumesophageal connectives and the brain all behaved qualitatively like an extract of the total nervous system. However, this seemed not to be true for extracts of the tritocerebral commissure.

In two experiments sea water extracts of eyestalks, tritocerebral commissures, and sea water were injected into three groups of overnight-dark-adapted *Palaemonetes* while they received a ten-minute exposure to an illumination of 250 ft.-c. at about 6 A.M. The extract of eyestalks was of such concentration that an animal receiving a dose of 0.02 ml. received the equivalent of half an eyestalk or a quarter the equivalent of the complement of a normal animal. The extract of tritocerebral commissure was prepared by removing the brain and circumesophageal connectives from several animals in such a manner that the tritocerebral commissures were not damaged. As they were removed, these organs were placed in sea water in a Syracuse watch glass until the desired number had been obtained. The commissures were then carefully removed by severing them with a scalpel at their junctions with the circumesophageal connectives. They were then transferred to a microscope slide

with a minimum of water and triturated as completely as possible with a small glass pestle with sea water to yield a concentration such that an animal receiving the usual dose of 0.02 ml. would receive the equivalent of ¼ of a commissure.

Each extract, including sea water as a control, was injected into five groups of 10 animals. After the light exposure and simultaneous injection of the three groups the animals were returned to the darkroom and the course of dark-adaptation was followed by making distal pigment index determinations for 10 animals of each group at 30, 60, 90, and 120 minutes from the beginning of the light flash.

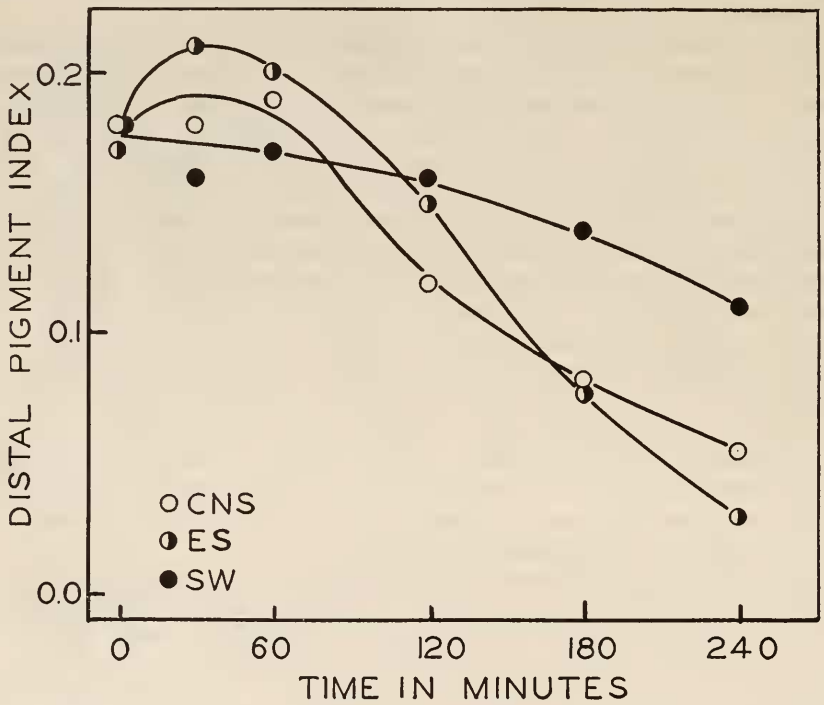

FIGURE 4. Comparison of the light- and re-dark-adaptational responses of prawns overnight in darkness to a simultaneous 250 ft.-c. light stimulus and injection of extracts or sea water. CNS—central nervous system; ES—eyestalk; SW—sea water.

It can be seen from Figure 5 that the injection of extract of tritocerebral commissure during the light flash resulted in a light-adaptational response which was significantly less than that achieved by the animals receiving only sea water. The animals receiving extract of eyestalks responded in a manner essentially similar to those in the previous experiments. The extent of light-adaptation was substantially greater than that shown by the sea water controls. These results suggested that in the tritocerebral commissure the dark-adapting hormone was present without the light-adapting one.

In order to establish further the character of the influence of the extract of the tritocerebral commissures a final experiment was designed. This one continued for a period of time long enough to follow re-dark-adaptation. In this experiment

three groups of three pans containing 10 animals in each were given a one-minute, 250 ft.-c. flash of light at 6 A.M. and then returned to the darkroom. At the end of an hour in the dark when the animals were expected to be maximally light-adapted as a result of the flash, they were brought into 250 ft.-c. illumination again for ten minutes during which time one group was injected with extract of tritocerebral commissure, the second group with an extract of eyestalk, and the third group with sea water as a control. The extracts were prepared in the usual manner. Following injection the three groups of pans were returned to the darkroom and distal pigment indices of 10 animals in each group were determined at 30, 60, 150, 210 and 270

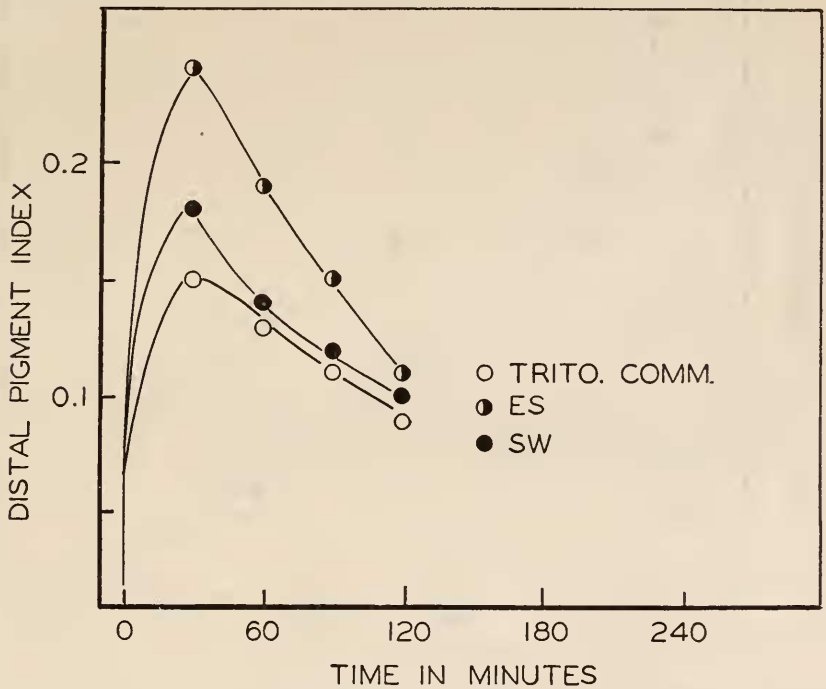

FIGURE 5. Comparison of the light- and re-dark-adaptational responses of prawns overnight in darkness to a simultaneous 250 ft.-c. light stimulus and injection of extracts or sea water. Trito. Comm.—tritocerebral commissure; ES—eyestalk; SW—sea water.

minutes following the beginning of the second light period. Since there were only three containers of animals in each group, each container was returned to the darkroom after each distal pigment index determination so that it could be used for another determination. Thus, container 1 was used for the 30-minute and the 210-minute determination while container 2 was used for the 60-minute and the 270-minute determination. It had previously been determined that the brief exposure to the microscope light during a determination for animals in this experimental state was not significantly effective in inducing light-adaptation. This is substantiated also by the response of the control group of this experiment.

Averages of the distal pigment indices of the 10 animals for each time that a determination was made were calculated and plotted in Figure 6. Zero time in this

figure indicates the beginning of the second light period. The probable index for all three groups at this time was estimated by extrapolation of the control curve.

It can be seen from Figure 6 that the influences of the extracts of tritocerebral commissure and eyestalks are qualitatively the same as in the previous experiment. Tritocerebral commissure depresses while eyestalk supplements the degree of light-adaptation following equal light exposure. However, the rate of dark-adaptation of the animals receiving extract of tritocerebral commissure is substantially the same as for those receiving extracts of eyestalk, and, furthermore, this rate is much

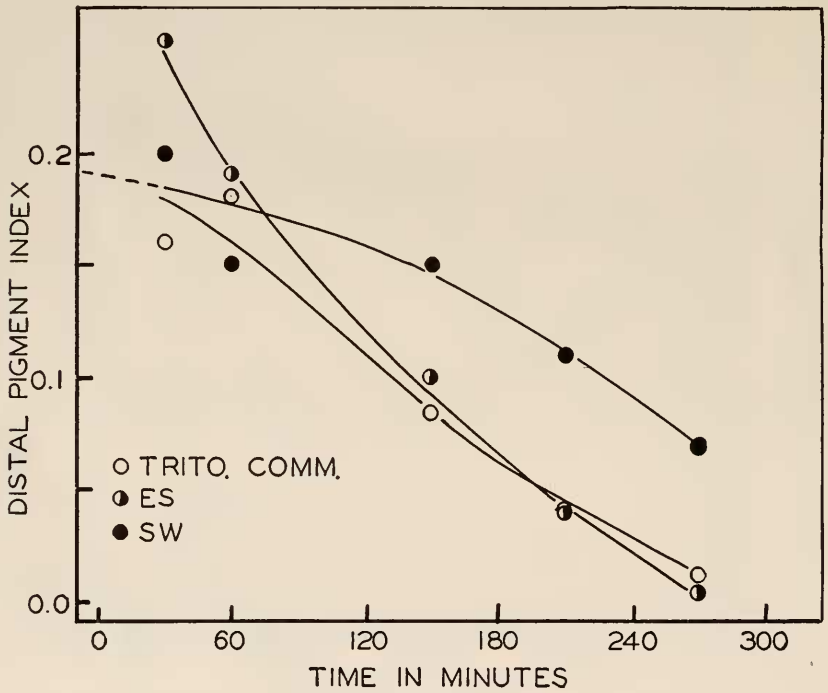

FIGURE 6. Comparison of the light- and re-dark-adaptational responses of prawns overnight in darkness to a simultaneous 250 ft.-c. light stimulus and injection of extracts or sea water. Trito. Comm.—tritocerebral commissure; ES—eyestalk; SW—sea water.

greater than that shown by the sea-water controls. Dark-adaptation of the two experimental groups was essentially complete in 270 minutes while the sea-water controls were still significantly light-adapted (distal pigment index of 0.068) at this time. It appears, therefore, that the tritocerebral commissure contains the dark-adapting principle but no significant amount of the light-adapting principle, while the eyestalks contain both principles.

DISCUSSION

The experiments involving the comparison of responses of animals with a single eye and eyestalk with those of normal animals clearly indicated that both eyestalks

are essential to the normal regulation of the retinal pigment of a single eye. This phenomenon appears to be reasonably interpreted, if taken as an isolated observation, in terms either of (1) loss of one of the two major photoreceptors of the organism or (2) loss of an eyestalk source of an endocrine factor concerned in light-adaptation. In terms of the former, there would be expected to be a reduction in the number of afferent pathways activated by light and consequently of impulses passing into the central nervous system. There could conceivably, therefore, be a reduced excitation of any endocrine gland located anywhere in the body in response to any given intensity of illumination. Although this possibility is a real one, it is rendered less probable by the observation that removal of one eyestalk and eye does not significantly alter the rate of dark-adaptation of the remaining eye in response to a light-to-dark change.

The alternative interpretation, that the removal of one of the stalks has removed a major source of a light-adapting hormone, is equally likely, and this one has the added support that it is completely consistent with the view held for several years that the sinus glands of the eyestalks are the most important sources of a light-adapting hormone for the distal retinal pigment. This view is also given support by the experiments involving injection of extracts reported in this paper. The eyestalks are far more effective than any other organ of the body in light-adapting action.

By similar reasoning, using the failure of the removal of one eyestalk to alter the rate of dark-adaptation of the remaining eye, there is support for the view that the eyestalks are not, relatively, as important sources of a dark-adapting principle. This also is strengthened by the results of injection experiments.

The work reported here has substantiated earlier work (Brown, Fingerman and Hines, 1952) that following one complete discharge of light-adapting hormone, some hours are necessary before there is a regeneration of the capacity to respond as strongly again to an equivalent stimulus. Here it seems quite evident that the endocrine sources become recharged through synthesis or accumulation of the hormone and discharge it again only in response to appropriate stimulation. This conclusion appears quite secure in view of the fact that the light-adapting hormone exercises a dominance over the dark-adapting one. That is, when both are present in substantial titers, the light-adapting principle appears to exert an action which is only very slightly depressed by the presence of dark-adapting hormone. Only when the conditions are such that there is a reduction in the quantity of light-adapting hormone present can the dark-adapting hormone exert that action which is then a function of its concentration.

On the other hand, the interpretation of the increase over some hours in darkness of the capacity to re-dark-adapt following the light-adaptational response to a one-minute, 250 ft.-c. flash is more difficult because of the aforementioned dominance. There may be either (1) a recharging of the endocrine glands concerned with the production of the dark-adapting hormone which is later discharged in response to the light-to-dark change which terminates the light flash, or (2) there may have been a gradual increase in blood titer of a dark-adapting hormone having such properties that it disappears only slowly from the blood over a period of some hours.

There is still too little information to permit one to reach a decision as to whether the dark-adapting hormone becomes stored in the glands of its origin in darkness.

The results do suggest, however, that there is no accumulation of light-adapting hormone in the sources in animals which are maintained for some hours in constant light at 250 ft.-c. Such animals, immediately following dark-adaptation during which it is presumed there would be no induced liberation of light-adapting hormone, have but little power of responding to a brief light flash by light-adaptation. On the other hand, there is the suggestion that the sources of dark-adapting hormone are provided with accumulated dark-adapting hormone when they are taken from constant illumination or during the day from the laboratory tanks where they have been subjected to the normal daily variation of illumination. Such animals can dark-adapt rapidly. However, immediately following induced dark-adaptation, these animals are unable to respond to a light-to-dark change with other than a very slow rate of re-dark-adaptation of their distal retinal pigment.

As was done by earlier investigators, attempts were made during the course of this work to induce dark-adaptation of the distal retinal pigment in light-adapted animals maintained in light by injection of various extracts. It might be presumed from the work reported herein that extracts of tritocerebral commissures would have yielded such results, but this was not the case. There are two reasonable explanations for such failure. One of these is to be seen in the dominance of the light-adapting hormone and the fact that all of the experiments were performed at intensities of illumination producing complete or nearly complete light-adaptation. At such intensities the state of the pigment would be expected to be determined predominantly by the light-adapting hormone. A second possible explanation is that the regulatory powers of the animals to illumination are such that the animals compensate for any alterations due to the injections by the secretion of more of the dominant light-adapting principle. It is interesting, in this latter connection, that the only condition under which it was possible in this work to demonstrate the presence of a dark-adapting hormone by injection was under the environmental condition of complete darkness. This condition would be expected to call forth maximal reduction in the blood titer of light-adapting hormone, and furthermore this is a condition to which there could not be expected to be any active adjustment.

Finally, it is evident from the experiments which have been described that, at least in darkness, injected light-adapting hormone disappears more rapidly from the blood than does injected dark-adapting hormone.

Summary

1. After removal of one eyestalk the distal pigment of the intact eye of *Palaemonetes* light-adapts more slowly and to a less extent for a given illumination; dark-adaptation is unaffected.

2. The eyestalks are the chief sources of light-adapting hormone; lesser amounts are found in brain, connectives and ventral ganglia.

3. The eyestalks and central nervous organs are sources of dark-adapting hormone. The tritocerebral commissure possesses dark-adapting, but no light-adapting hormone.

4. Both light- and dark-adapting hormones can be elaborated and stored, to be discharged in quantity upon appropriate stimulation.

5. Light-adapting hormone is elaborated and stored during a few hours in darkness; no store appears to be present in prawns kept in light.

6. Dark-adapting hormone appears to be stored in animals in light; following transfer to darkness, the store is depleted but the capacity to re-dark-adapt following response to a brief light flash gradually increases during a few hours in darkness.

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